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The Sixteenth Midwinter Conference of Immunologists was held January 22-25, 1977, at Asilomar Conference Grounds, Pacific Grove, California, with Joel W. Goodman and Leon Wofsy serving as Cochairmen. The subject of the conference, "Structure and Specificity--Antibodies and Receptors," was discussed during four half-day sessions by invited speakers. One evening of the conference was devoted to workshop programs, consisting of six working sessions held concurrently, but in proximity, to allow participants to move from one session to the other. There was no restriction on attendance, and a deliberate attempt was made to keep the workshops informal to encourage the exchange of ideas and information between workers engaged in related work. The workshops were organized by Claudia Henry (University of California at Berkeley) and J. D. Watson (University of California, Irvine).

Topics of the individual workshops were: (1) identification and significance of surface immunoglobulin on B and T lymphocytes; (2) isolation and molecular characterization of surface antigens; (3) use of surface markers in lymphocyte differentiation; (4) separation of lymphocyte subpopulations and functional analysis; (5) molecular techniques for detecting antibody genes; and (6) how membranes transmit signals.

A second evening of the conference was devoted to the Third Annual Dan H. Campbell Memorial Lecture and a speaker provided by the Graduate Students and Postdoctoral Immunologists Association. Their speaker was Dr. Laura Nader from the University of California at Berkeley. Dr. Nader spoke on "An Anthropologist's Look at Scientists."

The Third Annual Dan H. Campbell Memorial Lecture was given by Dr. Elvin A. Kabat (Columbia University, New York). The lecture was preceded with a few

introductory remarks by George Feigen (Stanford University), a close associate and friend of Dr. Campbell for many years. Joel W. Goodman (University of California, San Francisco) introduced Dr. Kabat, whose topic for the evening was "The Structural Basis of Antibody Specificity."

The problem of understanding the basis on which the very large number of antibody combining sites are formed cannot be solved by examining the thousands of different combining sites. One must approach the problem by making predictions about site structure and then attempting from sequence and x-ray crystallographic methods to verify the predictions and thus, by a series of successful predictions, elucidate the problems of site complementarity. A series of examples were given. They include: 1) the recognition of cavity and grooved type sites from quantitative immunochemical studies on anti-dextran myelomas, 2) the recognition of the three hypervariable regions of light and heavy chains as the complementarity determining segments with the rest of the molecule serving as a framework, so that predictions of site structure depends essentially upon residues in these hypervariable segments, 3) the use of paring analysis to locate contacting and structural elements in the complementarity determining segments, 4) identification of structural elements in complementarity determining regions from the distribution of the individual amino acids at these positions, 5) examination of similar sequences of complementarity determining segments of various proteins to determine contacting residues associated with differences in specificity. The implications of the occurrence of identical complementarity-determining segments in chains of different subgroups to the genetics of the generation of diversity was considered.

The first session of the conference, convened by J. D. Capra (Southwestern

Medical School, Dallas), dealt with the antibody molecule. Dr. Allen Edmundson (Argonne National Laboratory), the first speaker, spoke on a comparison of three-dimensional structure of eight chain Isomers in Mcg Immunoglobulins.

In the Mcg IgG1 immunoglobulin, the two light chains are noncovalently associated with heavy chains, but are covalently linked by an interchain disulfide bond. These two light chains have identical conformations, as evidenced by the presence of a crystallographic twofold axis of rotation between the L-H halves of the IgG1 molecule. In a Bence-Jones λ-type covalent dimer from the same patient, the two light chain monomers have strikingly different conformations, one resembling the Fd region of a heavy chain, and the other similar to the light chain component of a Fab fragment. After cleavage and reversible modification of the interchain disulfide bond, the light chains were dissociated from the heavy chains of the IgG1 protein and reassembled in to covalent dimers of conformational isomers like those in the Bence-Jones dimers. The reassembled dimers crystallized in two forms, the trigonal form characteristic of the native Bence-Jones dimer, and a needle form composed of altered dimers. Dissolved needles were indistinguishable from native dimers in chromatographic and electrophoretic behavior, but were markedly different in CD spectra. The spectral differences were interpretable in terms of the atomic model of the Bence-Jones dimer. The appearance of the needle form was subsequently taken as evidence for conformational changes resulting from dissociation or chemical modification of both light chain and Bence-Jones dimers. Noncovalent dimers, which were intermediates in the dissociation and reassembly experiments, were crystallized in the trigonal form and compared with native covalent dimers

by difference Fourier analysis. Solution and crystallographic techniques were used in combinations to define the type of conformational changes accompanying dissociation and reassembly of dimers, cleavage and reoxidation of the interchain disulfide bond, and the binding of bis (DNP)-lysine in the combining sites between the V domains. Procedures were devised to reverse these conformational changes in solution, and to crystallize the products for x-ray analysis.

Dr. Stuart Rudikoff (National Institute of Health) next described primary structural comparisons of phosphorylcholine, galactan, and levan binding myeloma proteins. Several groups of BALB/C myeloma proteins have been identified which bind the same haptenic determinants. In an attempt to assess the pattern of structural variation among proteins with similar antigen binding specificity, he performed amono acid sequence determinations on phosphorylcholine, galactan, and levan binding myeloma proteins. Four phosphorylcholine binding proteins have been studied in considerable detail. The light chains from two of these proteins (T15 and S107) have identical amino terminal sequences, while the light chains from M603 and M167 belong to different subgroups. Complete heavy chain variable region sequences have been obtained for all four proteins. Two of the heavy chains (T15 and S107) are completely identical. M603 is identical to the T15-S107 pair in the framework but has sequence and size differences in the complementarity regions. M167 has amino acid substitutions in the framework, as well as sequence and size differences in the complementarity regions. Studies on induced anti-phosphorylcholine antibodies in A/J mice have shown that the same sequences found in the BALB/C myelomas are also present in the strain.

In contrast to the phosphorylcholine binding proteins, light chains from five levan binding proteins all belong to the same subgroup. Among three nearly

complete variable region sequences, substitutions occur at five positions, two of which are in complementarity regions. Complete variable region sequences for four or five heavy chains show the complementary regions in all four to be identical with H3 being unusually short, consisting of a single amino acid. Six substitutions are found scattered throughout the framework, and based on the occurrence of these same amono acids in other heavy chains, suggest the possibility of more than one gene coding for these proteins. Light chains from a third group of antigen binding proteins (those with activity to β1 → 6 galactan) also belong to the same subgroup as in the case of the anti-levan light chains. Substitutions have been found at five positions, four of which are in complementarity regions. The single framework position showing variation (position 100) has three different amino acids in three separate light chains, all of which are found in other kappa light chains. This finding again suggests the possibility of either multiple variable region genes or the possibility of several constant region isotypes since it is not known whether position 100 lies in the variable or constant region.

Partial sequences from heavy chains of three of the galactan binding proteins show a pattern of diversity intermediate to the anti-phosphorylcholine heavy chains (which have complementarity region sequence and size differences) and the anti-levan heavy chains (which are invariant in the complementarity regions). Two of the proteins are identical in the framework portion and have two substitutions occurring in complementarity regions. The third heavy chain has three substitutions in complementarity regions and three framework substitutions in the C-terminal portion of the variable region.

The patterns of variation observed in these groups of myeloma proteins,

while neither clearly establishing nor refuting any of the major theories of antibody diversity, does place important constraints on several of the proposed mechanisms. Thus, any theory of antibody diversity must account for the types of variation observed in these groups of proteins which are in turn coded by genes of a single haplotype.

The next paper by Richard L. Wasserman and J. Donald Capra (University of Texas Health Science Center, Dallas) was presented by Dr. Wasserman and discussed the use of primary immunoglobulin structure as a probe of antibody function and evolution.

Extensive amino acid sequence studies of two monoclonal canine immunoglobulins (IgAκ Gom IgMλ Moo) have been performed. The proteins were products of spontaneously occurring lymphoid tumors of dogs.

The variable region of the Gom light chain was sequenced and shown to be more strongly homologous to human V_KII protein than human light chains of other subgroups. This is the first complete sequence of a nonhuman kappa variable region of the V_KII subgroup. This finding suggests the the V_K subgroup genes were present at the time of mammalian speciation.

The heavy chain variable regions of Gom and Moo were sequenced. These proteins are clearly assignable to the V_HIII subgroup. A deletion in the second hypervariable region of Moo was necessary to maintain homology. Neither Gom nor Moo exhibited phylogenetically associated residues beyond the third hypervariable region. These findings, taken with a sequence of a rat immunocytoma protein of the V_HII subgroup and the A/J anti-phenylarsonate antibody which is a V_HI, confirmed that genes encoding the heavy chain variable region subgroups were in the genome of the mammalian progenitor at the time of speciation.

The Fc of the canine muchain Moo was sequenced allowing the first inter-species comparison of a non-gamma heavy chain constant region. The overall homology of canine and human Fc is 80%. This degree of similarity is substantially more than that found for gamma or light chain constant regions of different species. Comparison of the canine and human proteins in the hinge regions reveals only 65% homology while the portion of the Cμ4 within the inter-chain disulfide is 90% homologous and the C-terminal 39 residues beyond the disulfide loop are 87% homologous. These data indicate that different heavy chain constant regions are preserved in evolution to a different extent and that portions of the muchain are extraordinarily conserved.

The first session concluded with a paper by Albert Nisonoff and Shyr-Te Je (Brandeis University) on the evidence that the B-cell repertoire is generated by a random process of somatic mutations. The paper was presented by Dr. Nisonoff.

Innoculation of rabbit anti-idiotypic (anti-id) antibodies suppressed the subsequent appearance of a cross-reactive idiotype (CRI) associated with the anti-p-azophenylarsonate (anti-Ar) antibodies of A/J mice. Such suppressed mice produced normal concentrations of anti-Ar antibodies which lacked the CRI, but against which anti-id antisera can be prepared. The anti-Ar antibodies of an individually suppressed mouse do not in general share idiotype with anti-Ar antibodies of other A/J mice, either suppressed or nonsuppressed. The present experiments were undertaken to quantitate several "private idiotypes" in a large number of hyperimmunized A/J mice. Anti-Ar antibodies of three mice, suppressed for the CRI, were labeled with ^{125}I and subjected to isoelectric focusing. Four single peaks, that were over 90% reactive with autologous anti-Id, were

randomly selected for use as ligands in a radioimmunoassay, and ascitic fluids containing anti-Ar antibodies from 181 A/J mice were tested as inhibitors. Two of the four idiotypes could not be detected in any mouse other than the donor. The concentration of the idiotype was less than one part in 1,250 to less than one part in 25,000 of the anti-Ar antibody population; these are minimum values. A third idiotype was detected in three of the 181 mice, but at a very low concentration. The fourth idiotype was present in 28% of the mice, again at a low concentration. The date supports existence of a very large repertoire of anti-Ar antibodies in the A/J strain and are consistent with a process of random somatic mutations for generating diversity in hypervariable regions. It is proposed that the cross-reactive idiotypes may be controlled by a germ-line gene or a gene related to a germ-line gene through a small number of somatic mutations; and that the idiotypes that were not detectable in other mice were the products of genes that had undergone extensive mutation, with a low probability of recurrence in other mice.

The second session on immunoglobulin genes, with G. Smith (University of Missouri, Columbia) as Chairperson, opened with Lee Hood (California Institute of Technology) who discussed the organization and evolution of the antibody genes.

Immunology has moved through a series of phases with respect to antibody molecules and antibody genes. At the turn of the century the basic components of the systems were defined. In the 1920s and 1930s many became impressed with exquisite specificity of antibody molecules. In the early 1960's molecular immunology came of age with protein chemistry, genetics, and serology. In the past year or so we have moved into a new era -- the era of nucleic acid chemistry of antibody genes.

Dr. Hood reviewed the major findings about antibody genes that came from protein chemistry (V and C regions, homology units, hypervariable regions, subgroups, and genealogical trees), genetics (three antibody families, V and C genes, V_H gene mapping), and preliminary nucleic acid chemistry (few germ-line genes per subgroup, germ-line genes for each subgroup). It was then pointed out the various ways that nucleic acid chemistry could solve certain problems in the area of antibody diversity, antibody gene expression and antibody evolution.

Dr. Hood concluded by pointing out that there are many other complex multigene systems, such as the nervous system, that have problems of "information handling" similar to those of the immune system. Thus, our analysis of immunology will lead the way for many other fields.

The second speaker, Ursula Storb (University of Washington, Seattle) discussed the occurrence of immunoglobulin mRNA like sequences in B and T cells. Using molecular hybridization techniques, she found that T cells contained immunoglobulin mRNA like sequences. Further analysis of κ- and λ-mRNA in B and T cells of tumorous and normal cell populations were also discussed. Complementary DNAs (cDNA) were prepared by transcribing mouse myeloma κ- and λ-mRNAs with reverse transcriptases. The cDNA corresponds to the untranslated 3' portion and part or all of the C region of the L-chain mRNAs. The κ-cDNA probe reacts fully with the RNA extracted from spleen, thymus, and all tumors of B and T cell origin which were tested. The amount of κRNA in various tissues was determined by comparing the cDNA hybridization kinetics of each RNA with pure κmRNA. The quantities varied by a factor of 8,000 with myeloma tumors containing a maximum and immature T cell lines a minimum. The κRNA was also found in the isolated cytoplasm of two T cell lines, suggesting that it may be translated in these cells. One leukemic cell line of neither B or T cell characteristics and a mastocytoma were

found to have relatively large quantities of κRNA in the nucleus, but none in the cytoplasm. Both B and T cell lines lost the capacity to synthesize RNA after continuous maintenance in tissue culture. Also, in general, the quantities of κRNA produced in newly established cultures were smaller than in tumors carried in mice.

Mouse thymus was found to have over 100 times less λRNA than κRNA. The κRNA in thymus T cells was translatable in cell-free protein synthesis into a number of different κ-chain precursors of a similar pattern as spleen κ-chains.

The sequence of κRNA in B and T cells was compared by thermaldenaturation of hybrids between κcDNA and the RNAs from various T and B cells. Under controlled conditions, identical melting points were found. It was concluded that at least the untranslated sequence of κmRNA and most of the C region is identical in T and B cells.

Janet Stavnezer (Sloan-Kettering Institute) reported on work by herself and J. Michael Bishop, concerning hybridization studies with DNA complementary to the variable region of immunoglobulin κ-chain. Dr. Stavnezer and associates prepared radioactive DNAs complementary to nucleotide sequences in coding the C and V regions of the immunoglobulin κ-chain produced by the mouse myeloma MOPC 41. Their procedures exploited two technical innovations. First, they used random oligodeoxynucleotides to initiate transcription of DNA from κ-chain messenger RNA by RNA-directed DNA polymerase. Since initiation occurred at various sites along the messenger RNA, this procedure circumvented problems encountered when DNA synthesis was initiated by oligo (dT) bound to poly (A) at the 3' terminus of the messenger. Second, a fractionated C region cDNA from V region cDNA by molecular hybridization with messenger RNA for the kappa chain produced by the NP2 variant of mouse myeloma; this RNA contains a deletion affecting the entire

V region and, therefore, hybridizes only with DNA complementary to the C region.

They characterized the C region cDNA and V region cDNA by molecular hybridization with RNAs from a series of mouse myelomas synthesizing κ-chains with different V regions. C region cDNA hybridized extensively with all of the RNAs tested. By contrast, the divergence of the V genes for the various κ-chains was manifest in the extent and rate of hybridization with V region cDNA and in the thermalstabilities of the hybrids. They estimate that a specific V region cDNA can anneal appreciably with only a relatively small fraction of the DNA encoding identified V regions in mice.

To determine the reduction in rate of hybridization of the V region cDNA with the average kappa V gene, the V and C region cDNAs were hybridized with total cytoplasmic RNA from mouse spleen. Spleen RNA probably does not contain transcripts for all kappa V genes, but it should represent the divergence of the average kappa V gene from the MOPC 41 kappa V region. The rate of hybridization of a V region cDNA was 32 times slower than the rate of hybridization of kappa C region cDNA due to the mismatching between MOPC 41 kappa V region and other kappa V regions. When the V region cDNA was annealed with excess total spleen DNA in the presence of ^{14}C- labeled unique sequence DNA, the rate of annealing of the V region cDNA indicated that they could detect 1.9 V kappa genes per haploid genome. By using the correction factor for the effect of mismatching on rate of hybridization, determined by the spleen RNA-cDNA hybridization experiments, and by examining the thermal stabilities of the hybrids, they estimate that there are between 60 and 130 κV genes in the mouse genome.

The last speaker in the second session was David Swan (National Institute of Health) who presented studies by himself, T. Honjo, and P. Leder on evidence concerning the genetic representation of mouse λ immunoglobulin sequences.

Molecular hybridization experiments designed to study the mechanism of

generation of antibody diversity rely on the accurate assessment of the number of constant and variable region genes in the genome.

Ititial hybridization experiments were performed with a complementary DNA (cDNA) made on mRNA extracted from a κ-producing tumor (MOPC-41). This cDNA was relatively short and represented only the C region of the κ light chain. Conditions were later established for the synthesis of cDNA representing the full length of the mRNA. With this full length cDNA it was possible to measure the hybridization between different κ-variable region sequences. MOPC-41 cDNA was found to form a complete hybrid only with its own mRNA. The variable region sequence did not form a stable hybrid with any of the other κ-mRNAs tested. In hybridization experiments with genomic DNA this cDNA would therefore only be expected to detect a MOPC-41 V-region sequence. In order to assess the total number of variable region sequences for a class of light chain, it was decided, therefore, to turn to the λ system, where the V-regions show much greater homology. Full length cDNA made on RPC-20 mRNA was found to hybridize completely to all other λ-mRNA's tested. RPC-20 cDNA could, therefore, be used as a probe to detect all known λ variable region sequences if present in genomic DNA.

RPC-20 cDNA was hybridized to mouse DNA from many different cell types, including sperm, and was found to exhibit unique kinetics over the entire range. This suggests strongly that there are only very small numbers (1-5) of λ variable and constant region genes in the mouse genome. This result is consistent with the number of genes that would be predicted by the somatic mutation hypothesis.

The subject of the third session, with Leon Wofsy (University of California, Berkeley) the Chairman, was "Antigen Binding Receptors on T and B lymphocytes."

Martin Raff (University of College, London) discussed antigen-specfic receptors on lymphocytes. Lymphocytes are like other differentiated cells in

that they are phenotypically committed to a particular functional program and the signals that occur on their surface initiate or modulate these functions. Unfortunately, lymphocyte responses are complex, involving cell interaction, proliferation, and/or differentiation and so it is impossible to study the molecular events that take place during cell signaling. What we have learned about signaling from studies of other cells suggests that surface receptors transduce extracellular signals into intracellular signals in two ways: By activating or inactivating membrane enzymes, or by opening or closing gated ion channels in the plasma membrane.

While it is known that immunoglobulin serves as antigen-specific receptors on B cells, it is still unclear what role they play in signaling the cell. In newly formed B cells, antigen or anti-immunoglobulin antibody binding to surface IgM invariably eliminates immunoglobulin from the cell surface and thus tolerizes the cell, but if T helpers are present, the cells are induced to make antibody. However, there is no evidence that antigen binding to immunoglobulin receptors on adult B cells can give a negative or a positive signal to the cell and in some situations it has been shown that B cells can be activated by ligands that bypass immunoglobulin receptors.

An important concept in viewing the immune response is that lymphocytes may be organized into networks based on idiotypes and possible allotypes and immunoglobulin class, and that antigen-induced responses occur by perturbing these complex networks rather than by "turning on" individual antigen-sensitive cells. While there is increasing evidence for idiotype networks, it still remains to be shown that anti-idiotype responses actually regulate normal immune responses.

There is increasing evidence that T cells require macrophages in order to respond to antigens and preferentially respond to antigens in association with

major histocompatibility complex (MHC) antigens. It seems likely that one receptor on T cells is a newly defined immunoglobulin heavy chain and that two independently coded receptors are used to recognize conventional antigens and MHC antigens. Even lectin-induced proliferation of T cells requires macrophages and in man there is now evidence that the required macrophage is Ia^+.

The next discussion was by Katherine Knight (University of Illinois) who discussed the presence of membrane IgD on murine B cells.

A goat antiserum and an allo-antiserum to mouse membrane IgG-like immunoglobulin have been prepared and the distribution of the IgD-like molecules was determined on mouse lymphoid cells by immunofluorescent studies. Double staining techniques revealved that IgD was present on nearly all (80-90%) of the IgM-bearing lymphocytes in adult spleen, lymph nodes, Peyers Patches. From 6 to 12% of the immunoglobulin positive cells (i.e., IgM and/or IgD) and IgM only and less that 3% had IgD only except in Peyers Patches, which had approximately 6% of the immunoglobulin positive cells with IgD only. With both anti-IgD reagents, half of the IgM-bearing cells in bone marrow also had IgD although the fluorescent staining was weak, presumable indicating a small number of IgD molecules per cell. IgD-bearing cells were also found in 4-day-old mice but again, the fluorescent staining was weak suggesting a small number of IgD molecules per cell.

No IgD plasma cells were found suggesting that the level of serum IgD would be small and, in fact, the fluorescent staining of membrane IgD could not be inhibited by mouse serum. Thus, IgD appears to be present in the mouse entirely on membrane and presumable exerts its function at the level of the membrane. This condition is different from that of the human in which concentrations of IgD range from 3-300 micrograms per ml but is similar to that found in monkeys where IgD plasma cells are also absent and serum IgD levels range from 30-100 ng.

The specificity of the two anti-IgD antisera were assessed by immunofluorescence and by reaction with ^{125}I-labeled lymphocyte membranes. By immunofluorescence, both reagents reacted only with immunoglobulin positive cells; cells capped with anti-immunoglobulin and restained with the anti-IgD reagents had double stained caps and no staining on the outside. Also, cells capped with either anti-IgD reagents did not co-cap IgM and conversely, IgM "caps" were not stained by either anti-IgD reagents. By reaction with ^{125}I-labeled lymphocyte membranes, the allo-antiserum reacted with a molecule which upon reduction and alkylation gave one band in the heavy chain region after gel electrophoresis in SDS; the mobility of this chain was faster than μ but slower than γ and is presumably δ. Incubation of spleen cells with goat anti-IgD resulted in the specific "stripping" of δ (IgD) from the membranes whereas μ (IgM) was not affected. Thus, both the goat anti-IgD and the allo-anti-IgD appear to be specific for IgD. The only possibility that the "anti-IgD" reagents are reacting with molecules other than IgD is that they react with a molecule specific to B lymphocytes and which co-caps with immunoglobulin when capped with anti-immunoglobulin but does not co-cap with IgM when capped with anti-μ.

The next talk was by Michael Julius (Basel Institute for Immunology). He discussed the expression of antiphosphorylcholine idiotypes of murine B and T lymphocytes.

Immunoglobulin receptors on phosphorylcholine (PC)-specific B cells bear predominantly the idiotype T15 which is characteristic of the Balb/c PC-binding myeloma protein TEPC 15. Antibodies directed to the T15 idiotype specifically inhibit induction of an anti-PC response. To determine whether "idiotypic homogeneity" is also characteristic of receptors on PC-specific T cells, he developed a functional assay for PC-specific helper cells. Balb/c mice were immunized with

PC coupled to isologous myeloma proteins. Splenic T cells were transferred along with BSA-primed B cells into irradiated syngeneic recipients which were then challenged with PC-BSA. In this system the PC-specific T cells were able to help BSA-specific B cells to give an anti-BSA PFC response. Their helper activity was completely inhibited when the irradiated recipients were injected with anti-T15 antibodies prior to cell transfer. Furthermore, he has been able to prime PC-specific T cells by injecting Balb/c mice with low doses of anti-T15 antibodies. Therefore, receptors for PC on T cells, like those on B cells, bear predominantly the T15 idiotype.

Injection of Balb/c mice at birth with anti-T15 antibodies changes the idiotypic pattern of the anti-PC antibodies from T15-positive ($T15^+$) to T15-negative ($T15^-$). Does neonatal suppression of the T15 idiotype also induce a change in the idiotypic pattern of receptors expressed on PC-specific T cells? PC-specific T cells from $T15^-$ mice and BSA-primed B cells from $T15^+$ mice were transferred into $T15^+$ irradiated recipients which were challenged with PC-BSA. The anti-BSA PSC response obtained was not inhibited by pre-treatment of the irradiated recipients with anti-T15 antibodies. Thus, PC-specific T cells from neonatally suppressed mice bear $T15^-$ receptors. In addition, he concluded that the target of the anti-T15 antibodies is not a molecule passively absorbed on a T cell surface, either directly or via an antigen bridge; it is a functional receptor present on PC-specific T cells (from conventional Balb/c) which bears the T15 idiotype. This parallel behavior of PC-specific B and T cells from conventional and neonatally suppressed Balb/c mice suggests that the T15 idiotype is present in the repertoire of PC-specific T and B cells and that neonatal suppression of this idiotype induces the expression of T15-negative receptors in both lymphocyte lineages.

The third session terminated with Samual J. Black (Stanford University School of Medicine) who spoke on studies on the expression and function of mouse IgD.

Using alloantisera specific for mouse IgD and hetero-antisera specific for IgM in indirect immunofluorescence and fluorescence-activated cell sorter analysis, he was able to show that most splenic B lymphocytes in the adult mouse have plasma membrane bound IgM and IgD. The antigen binding specificity of these molecules is very similar or identical. Both molecules can move on the cell surface, either independently, if capped with antiheavy chain sera, or together if exposed to antigen. In addition, on any given cell of a mouse heterozygous for allotype both the μ and δ heavy chains have the same allotype. IgD is not secreted in the mouse.

Functional analysis of B cells expressing IgD demonstrated that lymphocytes participating in primary immune responses are IgD positive, as are primary IgM plaque forming cells (PFC). IgG memory cells arising after exposure to antigen fall into IgD positive and IgD negative pools. The amount of IgG memory found within the IgD negative fraction depends on the elapsed time after priming before the cells are examined. After two exposures to antigen, most, if not all, IgG memory cells are IgD negative. IgG PFC are also IgD negative.

These data indicate that the expression of membrane IgD is limited to a major subset of B cells, mainly, the immunocompetent and as yet unstimulated (not deliberately stimulated) cell and IgM plaque forming cells. Whether or not IgG memory cells arising early after exposure to the antigen continue to synthesize IgD or merely carry residual IgD remains to be seen. To date he has been unable to define any specific function for membrane IgD.

Howard Gray (National Jewish Hospital) convened the fourth session on

"Other Markers of Functional Significance on T and B Lymphocytes." The first speaker was Hugh McDevitt (Stanford University) who discussed genetic organization, expression, and function of the I immune response region.

Following the initial discovery that genes determining the level of immune response to specific antigens were linked to the mouse major histocompatibility (H-2) complex, and mapped between the H-2 D and S loci of the mouse MHC, a large number of functions have been localized to this genetic region. These include specific immune response (Ir) genes, specific immune suppression (Is) genes, the antigens eliciting the mixed lymphocyte culture (MLC), and graph versus host (GVH) reactions, and a new class of cell surface alloantigens -- the Ia (immune response region associated) antigens.

Systematic analysis of the Ia antigenic specificity carried by different inbred strains of mice and congenic strains carrying recombinant H-2 chromosomes derived from crossovers between the H-2 chromosomes carried by standard inbred strains has revealed that the I region is a complex region which can now be subdivided tentatively into at least five subregions designated as A, B, J, E, and C. Recent evidence has revealed selective expression of I region gene products on lymphocyte subpopulations. Thus, there is now evidence to indicate that Ia antigens coded for by the I-A region are expressed on both T cells and B cells, and some suggestive evidence to indicate that the antigenic determinants coded for by this region are expressed by T cells & are distinct and different from those expressed on B cells. No Ia antigens can currently be mapped to the I-B subregion. Ia antigenic determinants coded by the I-J subregion appear to be expressed primarily on suppressor T cells, and possibly on macrophages and are not found on B cells. Antigenic determinants determined by the I-(E,C) segment appear to be expressed on both T cells

and B cells, and quite possibly on macrophages as well.

The multiplicity of functions expressed in the I region, and the complexity of the Ia antigens has led to an attempt to relate the Ia antigens to these functions. It seems clear that stimulation in the MLC and GVH reactions are due to the Ia alloantigenic differences. Since a number of T cell traced factors have been identified which share the following characteristics: 1) Ability to replace helper T cells (helper factor), suppressor T cells (suppressor factor); 2) molecular weight in the 40 to 50,000 dalton range; 3) possession of Ia antigenic determinants mapping to the I-A (helper factors) or I-J (suppressor factors) regions; and 4) antigenic specificity as indicated by ability to bind antigen columns -- the evidence indicates that the Ia antigens are in some way involved in the structure of molecules which appear to have antigenic specificity and be capable of replacing T cells in specificially immunized interacting cell systems. Recent evidence also suggests that complex I region determinants are also expressed in macrophages and that the macrophage plays a key role in the expression of genetic control of the immune responsiveness.

The problem of whether the I region gene products constitute a true antigen receptor system -- one with microheterogeneity in amino acid sequence among this particular class of gene products -- or whether they influence the ability to recognize antigens in an indirect way by a relatively nonspecific protein-protein interaction between gene products of the I region and other T cell or macrophage surface antigen receptors can only be resolved by a detailed analysis of the amino acid sequennce of the different classes of I region gene products. Hopefully, such an analysis will also indicate the manner in which gene products coded for by different subregions of the I region determin different lymphocyte functions including helper activity, suppressor activity, and cellular interaction.

Analysis of Ia molecules by two-dimensional gel electrophoresis was discussed by Patricia Jones (Stanford University). Mouse lymphocyte H-2 and Ia glycoproteins were analyzed by two-dimensional acrylamide gel electrophoresis techniques. This technique separates proteins first according to their charge in isoelectrofocusing gels and then according to their size in SDS gels. Individual polypeptide chains from radiolabeled cells are resolved as discrete spots on autoradiograms of the gels, forming patterns which are characteristic of the proteins in the sample.

Two-dimensional gels of H-2K, H-2D, and Ia glycoproteins immunoprecipitated from ^{35}S-methionine-labeled cells reveal that these proteins exist in the cells as complex arrays of molecules heterogeneous in both size and charge. Lactoperoxidase-catalyzed radioiodination of lymphocyte surfaces only tag subsets of the total H-2 and Ia molecules with ^{125}I, indicating that some of the molecules may represent cytoplasmic precursors of the cell surface proteins. This theory is supported by the kinetics of labeling of various spots in ^{35}S-methionine pulse-chase experiments. The labeling patterns suggest that some of the molecular heterogeneity may be due to the sequential glycosylation of these molecules during the translocation to the membrane.

C3 receptors and functional subpopulations of B cells was the topic discussed by George Lewis (University of California, San Francisco).

The existence of T-dependent and T-independent B cells was probed by employing in vitro limiting dilution techniques. Two T-independent antigens, TNP-Ficoll and TNP-Dextran, stimulated the same B cell subset. This subset was different from that activated by a T-dependent antigen, TNP-HRBC. Thus, distinct B cell subsets are capable of responding to T-dependent and T-independent antigens. Interestingly, another T-independent antigen, TNP-LPS, stimulated the same B cell subpopulation as TNP-HRBC, suggesting that a single

B cell subset can be activated via alternate pathways.

The antibody response to T-dependent antigens may be mediated by different B cell subpopulations as well. When splenic B cells were fractionated into complement receptor positive (CR^+) and complement receptor negative (CR^-) populations, and were cultured with carrier prime T cells, it was shown that CR^+ B cells were sensitive to C3 depletion while CR^- B cells remained unaffected by similar treatment. Additionally, CR^+ B cells could cooperate across haplotype barriers in the primary response, whereas CR^- B cells could not. Preliminary mapping studies suggested that two complementing Ir genes may be involved.

Mitogen activation of T cell specific soluble products associated with human β-2-microglobulin was the subject of the next talk by Vernon Maino (National Jewish Hospital).

The synthesis and secretion of β-2-microglobulin (β_2m) were examined in normal and leukemic mitogen-activated human lymphocytes. These studies indicated that activated T cells were capable of secreting large molecular weight proteins associated with β_2m. Analysis of lactoperoxidase or metabolically labeled tonsil lymphocytes by specific immune precipitation and SDS acrylamide gel electrophoresis, revealed a single 45,000 dalton HL-A component associated with β_2m. Recent investigations from other laboratories that β_2m may also be associated with secreted biologically active products prompted us to examine β_2m in the culture supernatant fluids of mitogen activated lymphocytes. Maximal secretion of β_2m from PHA-activated cells internally labeled with ^3H-leucine was found 24-28 h following stimulation. Analysis of anti-β_2m immune precipitates on reduced SDS polyacrylamide gels revealed three components of 70,000, 45,000, and 25,000 daltons plus the 12,000 dalton β_2m. Furthermore, at least two of these polypeptides were secreted by purified T cells, the B cell enriched population, on the other

hand, did not secrete significant amounts of these products. Additionally, the secreted β_2m-associated proteins could not be immunoprecipitated with anti-HL-A or anti-Ia sera. Concanavalin A elecited a biosynthetic response similar to that observed with PHA; in contrast, pokeweed mitogen, which was capable of stimulating immunoglobulin synthesis, did not enhance production of β_2m or its associated components. Lymphocytes from patients with chronic lymphatic leukemia (CLL) and acute lymphoblastic leukemia (ALL) were also examined for their ability to synthesize and secrete these products. It was discovered that CLL lymphocytes secreted only a 25,000 dalton polypeptide associated with β_2m, suggesting this component may be a B cell product. In contrast, analysis of the T cell leukemia (ALL) demonstrated the presence of only a 70,000 and 45,000 dalton polypeptide associated with β_2m in the cell lysates of these cells. These studies suggest secreted β_2m may exist as a subunit of products other than the major histocompatibility antigens. The identification of these proteins and their relationship to T cell function is currently being investigated.

The conference terminated with Victor Nussenzweig (New York University) discussing C3 and FC receptors and their function.

Dr. Nussenzweig reported on the reexamination of the role of particle-bound IgG and C3 in phagocytosis of sheep erythrocytes (E) by monolayers of purified human monocytes and polymorphonuclear leukocytes (PMN). He concluded that two fragments of the C3 molecule, that is, C3b and C3d, can function as opsonins if the phagocyte has the appropriate membrane receptors. Monocytes, that bind both C3b and C3d, respond to both as opsonins. PMN, which do not bind C3d, respond only to particles opsonized with C3b.

C3 and IgG have separate roles in phagocytosis. IgG, through its F_c fragment, directly stimulates particle ingestion, but is relatively inefficient

at inducing particle binding. On the other hand, C3 primarily mediates the binding of the particle via complement receptors. A marked synergy exists between C3 and IgG in inducing phagacytosis. Thus, opsonization of the particle with C3 can be a necessary condition for particle ingestion, although by itself C3 does not trigger phagocytosis.

The opsonic effect of C3 can be mimicked by a variety of nonimmunologic agents which enhance binding of the particle to the phagocyte without directly stimulating ingestion. The contact-inducing agents used include centrifugation of particle and phagocyte, high molecular weight dextran, protamine, and treatment of E with neuraminadase. These results strongly suggest that the role of C3 in opsonization is mainly or exclusively one of establishing contact between particle and phagocyte.